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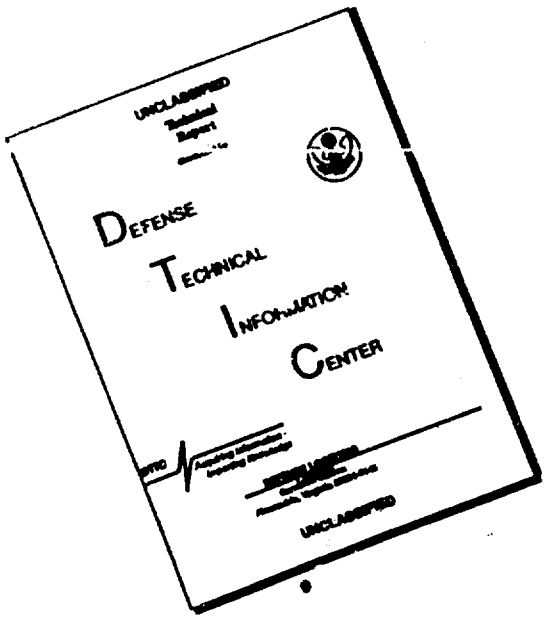

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Effect of Botuline Toxin B on Tissue Respiration in the Brain of Guinea Pigs

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Clinical manifestations of botulism are primarily disclosed by neuroparalytic symptoms (A.M.KORITSKII, 1937). Severe forms of the disease are distinguished by a sharp increase in bulbar occurrences, which may lead to a fatal end as a result of the respiratory center paralysis.

Pathologoanatomists point out disparities that develop in patients with severe functional disorders, but with relatively insignificant changes in pertinent sections of the central nervous system (A.P. AVTSIN et al., 1957). This may indicate to a certain extent that basic functional disorders in botulism are due to changes in most any vitally important biochemical processes. A. GUYTON and M. McDONALD (1947) regard the botuline toxin as a "destructive enzyme". K.I. MATVEEV (1958) traces it to antimetabolites.

Curariform action has been attributed to botuline toxin for a long time. However, evidence was discovered recently that the toxin possesses no such effects (A.S. BURGEN, F.DICKENS and ZAT-

MAN, 1949).

It has also been determined that the toxin has no effect on acetylation of choline and it does not change the activity of cholinesterase (C.TARDA and H.WOLFF, 1947; J.STEVENSON and G.GIRVIN, 1953).

The majority of foreign authors assume that the toxin exerts a peripheral action. They often note that the reason for development of muscular paralyses is either due to injuries of the motor nerve endings, or due to injuries in their immediate proximity (E.AMBACHE, 1951; J.DAVIES, 1953).

Considering rather numerous reports that clarify the mechanism of the botuline toxin's actions, there are but few findings pertinent to the toxin's effect on biochemistry of the central and peripheral neural systems. Hence, we decided to investigate the effects of botuline toxin type B on tissue respiration in the brain and in myelencephalon of guinea pigs.

We determined the absorption of oxygen and liberation of carbon dioxide using WARBURG apparatus and DICKSON'S method in modification of N.P.MESHKOVA and S.E. SEVERIN (1950). Observations were made every 30 minutes for 1½ hours.

The intensity of tissue respiration was expressed in microliters of absorbed oxygen and liberated carbon dioxide per 1 mg of dry tissues. In addition, we computed the respiration quotient, i.e. the relationship of liberated carbon dicxide to absorbed oxygen. In all, we performed 125 determinations on 92 guinea pigs.

Botuline toxin was administered intramuscularly to animals in doses of 1 Dlm and 100 Dlm for one guinea pig. The minimal fatal

dose for one guinea pig weighing 200 to 250 gm was equal to four mouse-Dlm. The activity of 1 mg of dry toxin corresponded to 500 Dlm for guinea pigs.

Animals that received 100 Dlm were killed after 20 minutes and those that received 1 Dlm were killed either after 20 minutes (1st series), 24 hours (2d series), or 48 hours (3d series).

Irrespective of the dose of toxin, 20 minutes after its administration, the general condition and behavior of animals showed no changes. Most guinea pigs that received 1 Dlm showed on the 2d day some decrease in muscular tonicity, while their general mobility remained unchanged. The condition of guinea pigs deteriorated considerably on the 3d day: muscular tonus declined sharply and the animals assumed a lateral position with their extremities stretched out.

Observations made by us indicated that botuline toxin in 1 Dlm dose 20 minutes after its administration caused increased liberation of carbon dioxide in brain tissues on the average by 18%, while the increase of carbon dioxide in tissues of myelencephalon reached 42% (see Table 1).

Changes in oxygen absorption by these tissues were less expressed; one could only note a tendency toward some increase. The tendency was more distinct in myelencephalon. The above condition was accompanied by an increase in the respiratory quotient by 24% in tissues of myelencephalon and by 12% in cerebral hemispheres.

Twenty four and 48 hours after administration of toxin, the increase of carbon dioxide in both these sections was somewhat reduced. Also reduced in these experiments was the absorption of

Table 1

Absorption of oxygen and liberation of carbon dioxide by brain tissues of guinea pigs following the administration of botuline toxin type B. Experimental conditions: 2 ml of phosphatic buffer solution, pH = 7.4; each test included 100 mg batch of pulpy brain tissues, 90 minute incubation period, 37°, in pure oxygen

Time of sight		Cerebral hemispheres									Myelencephalon								
		Absorption of O_2			Liberation of CO_2			Respiratory quotient			Absorption of O_2			Liberation of CO_2			Respiratory quotient		
		Control	Test	Deviation in %	Control	Test	Deviation in %	Control	Test	Deviation in %	Control	Test	Deviation in %	Control	Test	Deviation in %	Control	Test	Deviation in %
Following administration of 1 Dlm																			
After	20 min.	6.70 ±0.2	7.61 ±0.22	+1.6	6.45 ±0.33	7.63 ±0.18	+8	0.96 ±0.04	1.08 ±0.03	+12	3.21 ±0.21	3.68 ±0.25	+14.6	2.66 ±0.24	3.77 ±0.25	+42	0.82 ±0.07	1.02 ±0.04	+24
	24 hrs.	6.70 ±0.27	6.85 ±0.32	+2	6.45 ±0.33	6.81 ±0.29	+5	0.96 ±0.04	1.04 ±0.06	+8	3.21 ±0.21	3.36 ±0.32	+4.6	2.66 ±0.24	3.08 ±0.28	+15.8	0.82 ±0.07	0.91 ±0.11	+10
	48 hrs.	6.70 ±0.27	6.28 ±0.36	-6.2	6.45 ±0.33	6.92 ±0.29	+7	0.96 ±0.04	1.16 ±0.08	+20	3.21 ±0.21	3.46 ±0.21	+7.7	2.66 ±0.24	3.09 ±0.27	+16	0.82 ±0.07	0.86 ±0.10	+5
Following administration of 100 Dlm																			
	20 min.	6.81 ±0.59	6.44 ±0.33	-5.4	5.93 ±0.25	5.59 ±0.33	-5.7	0.87 ±0.09	0.93 ±0.07	+6.8	3.84 ±0.29	2.99 ±0.18	-21.8	2.37 ±0.19	3.75 ±0.54	+58	0.96 ±0.23	1.27 ±0.09	+32

oxygen by brain tissues, while we noticed in myelencephalon tissues (quite like in 20 minutes after inoculation) a trend toward an increase in the consumption of oxygen.

We noticed a tendency toward a decline in tissue respiration intensity in cerebral hemispheres 20 minutes after administration of 100 Dlm dose of botuline toxin to animals, also a decrease in the absorption of oxygen and in the liberation of carbon dioxide.

Yet, other changes developed in myelencephalon. Here, the absorption of oxygen decreased on the average by 22% and, vice versa, the liberation of carbon dioxide increased by 58%, i.e. more than after administration of 1 Dlm.

Thus, we discovered that botuline toxin causes changes in tissue respiration dissimilar in their intensity in cerebral hemispheres and in myelencephalon. The changes are more significant in the latter brain. This diversity in action is conformable with clinical and pathological findings. As we know, the bulbar syndrome reveals itself as prominent in the clinical picture of botulism and one can discover extreme morphological changes in cells of the truncal part of cerebral hemispheres, if compared with other sections of the brain (A.P. AVTSIN et al., 1957).

Respiratory stresses in examined tissues were primarily manifested by increased liberation of carbon dioxide and by a lesser increase in the consumption of oxygen (in some cases even by a decrease). Maximal changes were observed after 20 minutes following the administration of toxin. These changes were less distinct on subsequent days, including the 2d and 3d day.

The results of our experiments and of investigations of the

botuline toxin's type B effects on absorption of oxygen by brain tissues conform with the findings of BURGEN, who used the WARBURG method to examine the action of type A botuline toxin on white rats; he, likewise, failed to observe significant displacements in the absorption of oxygen by tissue sections of the brain.

The fact that,more significant changes we determined in the liberation of carbon dioxide than those found in the absorption of oxygen,obviously implies that impairments in decarboxylation processes are produced under the conditions of botuline intoxication.

It can be assumed that one of the reasons for the liberation of excessive carbon dicxide is the increased decomposition of keto acids, particularly of pyruvic acid, which appears to be a more important substrate in tissue respiration (N.F. MEDVEDEVA, 1957; M.I. PROKHOROVA, 1949). A verification of this should be attempted in subsequent investigations.

Conclusions

1. Botuline toxin in a dose of 1 Dlm and 100 Dlm affects the respiration of tissues in myelencephalon more than in cerebral hemispheres.

2. The administration of 1 Dlm of botuline toxin caused intensification of respiratory processes 20 minutes later in examined tissues; they were manifested by a predominantly increased liberation of carbon dioxide and by alvanced respiratory quotient.

3. Dose of toxin increased to 100 Dlm induced a decline in the level of respiratory processes in cerebral hemispheres.

The reaction in myelencephalon brought alongside with a decrease in oxygen absorption also an intensified liberation of carbon dioxide.

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